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REVIEW

POLYMER MAGNETIC PARTICLES IN BIOMEDICAL APPLICATIONS

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In this review, general methods of obtaining and characterization for magnetic particles, for magnetic polymer particles and for drug charged magnetic polymer particles are summarized. Drug release from magnetic systems, characteristics of magnetic carriers and clinical testing of therapeutically drugs are also illustrated. Some current biomedical applications (drug vectoring with magnetic carriers, antitumoral therapy by electromagnetically induced hyperthermia, locoregional radiotherapy with magnetic particles, erythrocytes' magnetic separation, contrast agents in magnetic resonance imaging) of magnetic polymer particles are reviewed. Possible perspectives are also discussed.

INTRODUCTION

Magnetic particles of small dimensions offer some interesting possibilities in drug targeting, due to their controllable size, from a couple of nm to tens of nm, comparable to those of the cell (10-100 μm), virus (20-450 nm), protein (5-50 nm) or gene (2 nm width and 10-100 nm length). They can be encapsulated in an entity of biological interest or can exist as an insertion material in a non-magnetic material – biocompatible polymers: dextran, xanthan, polylactide, gelatine.[1,2] As magnetic carriers, the most used are the ferrofluids because they present some advantages comparative to other magnetic carriers, such as: the perfect compatibility, the excellent stability *in vivo*, the migration in magnetic field.

One of the latest research directions is the covering of the magnetic particles with biodegradable and biocompatible synthetic polymers. The polymers, as cover layer of the magnetic sensitive particles, offer the possibility of adsorption and of the chemical binding to biologically active substances.

Drug administration by known ways leads to a uniform distribution in the body, (including healthy areas) of the active principle. In order to avoid this, the concept of "intelligent drug" has been introduced, which can be strictly directed to the sick area, by applying an externally magnetic field. The condition is that the drug is sensitive to the presence of the magnetic field, meaning that it is associated itself with the magnetic particles. Usually, both the drug and the magnetic particles are included in a polymeric matrix. In literature, such a system is called "drug delivery polymeric magnetic particles".

The first magnetic carrier for drug delivery was obtained by Widder,[3] in 1978 and consisted in albumin microcapsules (0,2-2,0 μm), containing Fe_3O_4 as magnetic particles of (10-20 nm) and adriamycine as therapeutic agent. The delivery in the body of the complex drug is made by intravenously or intra-arterially injections, whilst an external magnetic field is guide and concentrate the drug to the trouble area. The release of the therapeutic agent from the complex is either accomplished through enzymatic activity or by changing the physiological conditions (pH, osmosis or temperature). This system founded the development of other numerous similar magnetic polymeric drug delivery systems.[4-22] In 1995 Devineni[23] studies the methotrexate distribution administered through cerebral injection as solution and as magnetic microspheres. The conclusion is that once the exterior magnetic field is ceased, the microspheres are no longer retained in the brain,

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being redistributed in the whole body, especially in the liver.

For the first time, in 1996, using particles (100 nm) of a ferrofluid chemically bounded to epirubicin, Lubbe[22, 24] does some clinical tests on humans and obtains promising results. The conclusion drawn from here is that the bigger the magnetic particles and the higher their magnetic susceptibility is, they can be directed towards and retained in the target area easier.

MAGNETIC MICRO AND NANOPARTICLES

In general, magnetic micro and nanoparticles are made of substances with a very strong magnetic character (iron, iron oxides – magnetite, different ferrites), particles that present a strong magnetic moment. Such compounds may deliver nonmagnetic entities, like cells, active biological substances (antibodies, antigens, enzymes, nucleus acids, drugs), pathogenic agents, xenobiotics, etc into magnetic fields.

1. Iron – carbon particles

The heart of these particles is made of iron or iron oxides covered by a thin layer of carbon. This magnetic carrier is an excellent absorber due to the porous carbonic phase, which large specific surface favors the molecules to fix on peptides, proteins and drugs. The dimensions of the particles are between 0,01 μm and 1 μm. This kind of particles can be mainly obtained by two methods: (i) high energy ball milling of elemental iron and activated carbon particles[25,26] and (ii) the Kratschmer-Huffman carbon arc method.[27, 28] The advantage of the first method consists in the capacity of producing high porosity carbon covers, with a large absorbency surface for drugs. The method is used especially for preparing metastable and exotic combinations that cannot be obtained by the conventional technological methods.[29]

There are also other methods for obtaining them, like electrochemical,[30] ultrasound[31] or methods implying humid grinding of magnetite in vibrating mills with balls and metallic grinding vessels.[29]

2. Magnetic fluids

The magnetic particles capable of forming super paramagnetic dispersions in fluid carriers can be metals or metallic oxides with the diameter between 1 and 100 nm, such as Co, Ni, Fe, Fe_3O_4, γ-Fe_2O_3. In pure state metals have the highest susceptibility. Still, these transitional metals are very toxic and very sensitive at oxidation. In atmospheric conditions Ni, Co oxidizes into NiO, CoO, which are antiferromagnetic.

The magnetic fluids, also called ferrofluids, are made of nanoparticles of iron oxide, with dimensions around 10 nm, suspended in a carrier liquid that can be polar or nonpolar. The magnetic ferrofluids must be stable dispersions of ultrafine magnetic particles or encapsulated magnetic particles in an aqueous carrier medium. The stabilization of the particles can be done with surfactants, which stop their flocculation and sedimentation.

The most used methods are the following: co-precipitation, micro emulsification and de-composition of the organic precursors.

Co-precipitation is made between iron salts II and III, in alkaline medium. Bee, 1995,[32] obtained very small (2 nm) magnetite particles, by performing the precipitation in the presence of citrate ions. The most well known method is Massart's one, 1981,[33] which synthesized the aqueous magnetite ferrofluid by co-precipitation of ferrous and ferric chlorides in ammoniacal medium, in molar ratio 1:2, without adding tensioactive for stabilizing, but using a peptization phase as counter-ions. Shinkai[34] prepared magnetite nanoparticles oxiding ferrous sulphate with sodium nitrite, by precipitating with an ammoniacal solution and stabilizing with oleic acid; he noticed that the increase of the ratio Fe^{+2}/ NO_2^-, determines an increase up to 70 nm of the particles diameter. Sun[35] performed the partial reduction of the ferric chloride with natrium sulphite and the precipitation in ammoniacal medium, using the ratio Fe^{+3}/SO_3^{-2} of 3 obtaining particles with 7 nm diameter.

The principle of the **synthesis in microemulsion** water/oil (or reversed microemulsion) consists of forming some water micro drops dispersed in organic phase and stabilized with tensioactive molecules. This is how Feltin[36] prepared the 4-12 nm particles, using a ferrous dodecyl sulphate solution. The dimension of the particles depends on the working temperature and on the concentration of the tensioactive.

The method of decomposing organic precursors is performed at temperature using ferrous organic forerunners, in the presence of tensioactives. Nanoparticles with a narrow

distribution of diameters and high crystalinity are obtained. Hyeon[37] used iron pentacarbonyl treated with oleic acid at 100°C; the formed complex was decomposed in nanoparticles at 300°C, oxidized at maghemite with trimethylamine; diameters between 4 and 16 nm were obtained, depending on the ratio $Fe(CO)_5$/oleic acid.

The stabilization of the colloids is of great importance in the precipitation and stocking of the nanoparticles in fluid state. In aqueous phase, nanoparticles can be stabilized through ionic interactions, with an surfactant in double layer (for example, fat acids, aspartic and glutamic acids, meso – 2,3 – dimercaptosuccinic acid, peptides).[2] If the iron oxides magnetic particles are not covered, their surfaces are hydrophobic, and the ratio between surface and volume is high. For an efficient stabilization of the iron oxides particles it is necessary to cover almost all with surfactants.

Magnetic fluids can be used to prepare some types of particles, like: magnetoliposomes, biocompatible and biodegradable magnetic polymers that can be also used for isolating and separating the specific molecules,[66,67] to encapsulate drugs and radionuclides[38] or in the medical field in order to vector drugs magnetically.[10-22]

3. Methods of characterization

At present, from the magnetic particles, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are mostly used for biomedical applications. The characterization of the magnetic particles from the ferrofluid is performed through chemical dosage, when the concentration in iron oxide is determined; the stability of the ferrofluid is studied with the pH method; the dimension of the particles is determined by electronic microscopy of light transmission and dynamic diffusion; the specific surface is determined by nitrogen adsorption; the density of the hydroxyl groups is performed with the help of thermogravimetric analysis and triethylaluminium grafting; magnetic properties are studied using vibrating sample magnetometer; grain size is estimated by X-Ray diffraction. Mossbauer spectroscopy can give very precise information about the chemical, structural, magnetic and time-dependent properties of a material. Scanning electronic microscopy is used in the analysis of micro and nanospheres, especially for providing quality data like particle dimensions, surface rugosity, form of microspheres as well as other information (the existence of exceeding material, sample polydispersity, particles fragility – the presence of a great number of destroyed particles on field).

MAGNETIC POLYMER PARTICLES

The magnetic carriers (MC) are made from iron oxides or greigite particles (Fe_3S_4) dispersed inside a polymeric matrix; their surface can be modified so that different coupling methods can be applied. For the same purpose, silanized particles of iron oxides or magnetic porous glass can be used. The polymeric layer from the surface of the particle has the role of protecting the target cells from the possible exposure to the iron toxic action.

The magnetic carrier method consists of the selective – permanent or temporary – attachment to a microparticle with a high magnetic moment (the magnetic carrier, MC) of some nonmagnetic entities (EN), like as biologically active substances, subcellular elements, nucleus acids, pathogenic microorganisms etc, and vector (separation, directing or fixing) the formed groups through magnetic systems.[2]

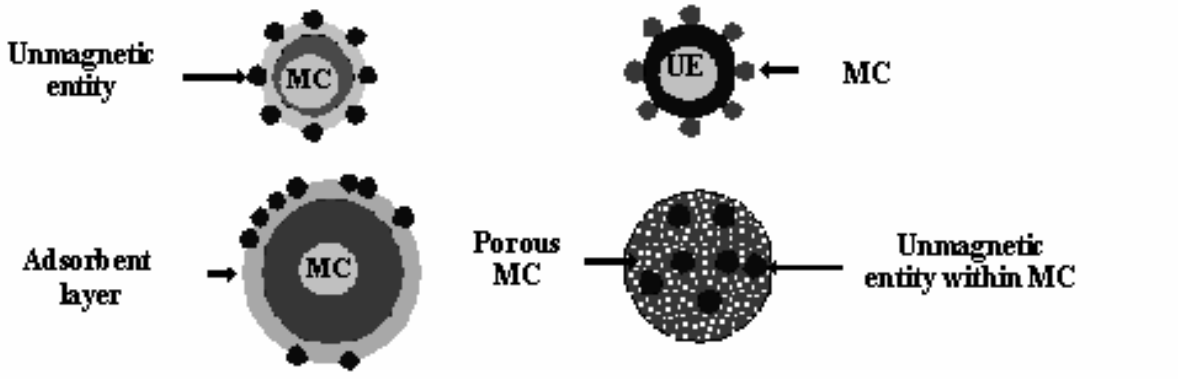

Fig. 1 – MC-EN coupling modalities.

Depending on the character of the target cells and the further operations, a couple of strategies can be chosen on how to create the magnetic carriers, (magnetic or super magnetic particles, magnetic fluids, magnetoliposomes or magnetotactic bacteria). Most of the times, the magnetic properties of the carriers are determined by the presence of Fe_3O_4 (magnetite) or $\gamma - Fe_2O_3$ (maghemite) nanoparticles, ferrite (cobalt ferrite Fe_2CoO_4) or chrome dioxide (CrO_2) particles being used in some cases.

The magnetic carriers can have one of the following configurations[40]: (i) a magnetic particle of magnetite or maghemite covered by a compatible polymer or (ii) a biocompatible porous polymer inside whose pores the magnetic particles have precipitated. More recently, the researchers concentrated their attention upon some carriers that contain either noble metals (Au), or alternative metals (Co, Ni, Ir).

The magnetic particles can be classified according to several criteria:

From the dimension point of view, there are the magnetic nanoparticles whose dimensions are between 5-100 nm (magnetoliposomes and magnetic ferrofluids can be included here) and the magnetic microparticles whose dimensions vary between 1-300 μm.

From the point of view of the carriers' wall structure, there are the magnetic carriers without cover, where the magnetic particles are in suspension into a delivery medium (magnetic ferrofluids of Fe_3O_4, $CoFe_2O_4$ and FeC can be included here; depending on the type of the delivery medium these ferrofluids may be classified: hydrophilic and hydrophobic) and the magnetic carriers with cover, who can be: with simple wall, with double wall (with a complex wall where the number of layers is higher than two).

Depending on the behavior in the biological medium, there are magnetic carriers with resorbable or biodegradable cover that can be digested by the enzymatic system of the live cells (this category includes biocompatible polymers: proteins, lipids, dextran, gelatin, poly(ethylene glycol), poly(ethylene oxide)-polyethylene, polylactic acid, poly(acrylic acid), poly(vinyl alcohol), aspartic acid, glutamic acid, etc.) and magnetic carriers with nonresorbable or nonbiodegradable cover: polyethylene, polyethylene terephthalat, polystyrene.

Depending on their nature, the magnetic carriers can be synthetic, after applying some synthesis techniques upon active magnetic particles (including the majority of magnetic carriers) and natural, produced in the shape of magnetosomes in the cytosole of the magnetotactic bacteria: *Magnetospirilum magnetotacticum, Aquaspirillum Magnetotacticum* (these are released from the cell only by destroying the cellular membrane).

A particular group of magnetic carriers includes the **magnetoliposomes,** which vary widely in dimensions depending on the technique approached for their synthesis; they can be included either in the group of the ferromagnetic liquids or in the group of the microparticles. As well as in the group of the synthetic carriers with biodegradable wall.

1. Methods of obtaining

The methods of obtaining the magnetic microparticles are: chemical and physical methods. Coprecipitating transitional metals that have free coordinating spaces, in the presence of a polymer creates magnetic polymeric particles; they are capable of forming stable aqueous suspensions that are easily re-suspended after agglomeration.

In the category of chemical methods are included: procedures that use polymerization and polycondensation of monomers: polymerization in emulsion, polymerization in dispersion, polymerization in suspension, interfacial polymerization, polycondensation in suspension, polycondensation in dispersion, precipitative polycondensation, as well as procedures that use preformed polymers in order to produce drug spherical carriers: the solvent extraction/evaporation method, the reticulation in suspension method, the coacervation, the precipitation, the kelatization, the melting solidification. The magnetic material is generally introduced in the reticulation phase, either as a magnetic fluid, or as magnetic powder.

2. Methods of characterization

The characterization of polymer microparticles with magnetic properties can be performed by: COULTER technique, for dimensioning and obtaining the dimensional polydispersity grade of the particles, scanning electronic microscopy (SEM), atomic force microscopy (AFM) and SQUID technique for analyzing the magnetic characteristics.[41]

The atomic force microscopy (AFM) is an analyzing technique of the surfaces of a rigid material up to the atomic level. It provides qualitative and quantitative information upon many physical properties including the dimension, (length, width, height), the morphology, the texture of the surfaces and rugosity. The statistic information like: particle counting, surface area, volume distribution could also be determined from AFM measures. Knowing the density of the material, the mass distribution of the particles can also be calculated.[42, 43] SQUID technique detects the magnetic moment of a material sample, from which magnetization and magnetic susceptibility can be later found.

MAGNETOLIPOSOMES

The magnetoliposomes are biocompatible structures, physiologically formed of phospholipidic vesicles that contain magnetic particles of nanometric dimensions, either in the lipid bilayer or in the aqueous compartment.

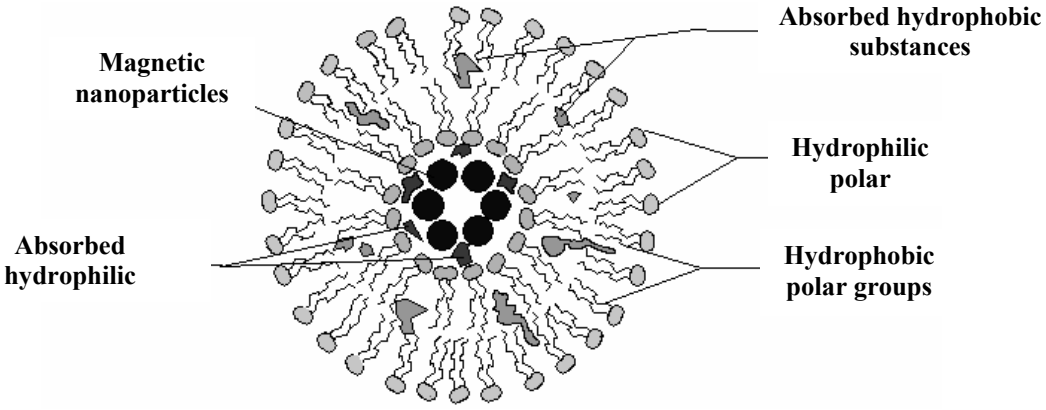

Fig. 2 – Structure of a magnetoliposome.

There are two kinds of magnetoliposomes (ML): one contains iron oxide particles dispersed in aqueous heart, and the other is formed of iron oxide particles of 15 nm, stabilized and solubilized with laureth and covered with lipid bilayer. Magnetic liposomes can be obtained by precipitating magnetic particles in the presence of phospholipidic vesicles (used as nanoreactors). The existence of the phospholipidic bilayer that surrounds the magnetic particles was confirmed by both TEM of the marked samples with uranyl acetate and digital imagistic measures in the fluorescent microscopy of the fluoresceine marked magnetosomes.[1,44]

Another method for obtaining the magnetoliposomes consists of covering the magnetite nanoparticles (10 nm) with phosphatidylcholine/ phosphatidylethanolamine in 2:1 ratio, resulting in some particle agglomerations with the diameter of almost 80 nm. In order to stabilize the phospholipidic capsules and to produce an "anchor" necessary to antibodies bondage, ML were covered in pullulan hydrazide.[45]

Incorporating a phospholipid functionalized derivate poly(ethylene glycol) in the phospholipidic cover of the magnetite has as result the obtaining of a membrane capable of fixing the water soluble proteins. The magnetoliposomes prepared from these phospholipidic molecules proved to be interesting colloids for evaluating the immobilization grade of the enzymes in a fast and elegant way.[46] Raman spectroscopy was used as an experimental evaluating technique of the interaction between the active chemical surface of the nanoparticles and the head of the polar group of the internal phospholipidic layer.[47]

One of the advantages towards liposomes is the sanguine circulation time, obviously more important, the magnetoliposomes being more difficult captured by the reticuloendotelial system (RES), especially towards liver and spleen. Another advantage is, besides the biocompatibility the lipid layer offers, the sensitivity to the action of the magnetic field. They are able to be directed, with the help of the magnetic force, towards the target organs and cells. The magnetoliposomes can go through the hematoencephalic barrier being used in the research upon brain tumor treatment.

The idea to link different molecules to the surface of the magnetoliposomes was put into practice using PEG molecules with biotin – attached to one of chain's ends; as many

streptavidinilate molecules (ligands and monoclonal anti-bodies) can be easily attached to it.[48] The same idea, to charge the magnetoliposomes with curara-like components which relax the peripheral muscles (diadone and diperone), largely used in anesthesia, was put into practice to obtain a selective relaxation of the muscles in a certain area of the body without affecting the respiratory muscles or the heart.[49]

These formations can be used for encapsulating different drugs and radionuclides with possibility of use in medical field in magnetic vectoring of drug (the main use being cancer therapy). Antibiotics, trombolytics, anti-inflammatories, peptides and steroids can be released in the same way.[1]

MAGNETOTACTIC BACTERIA

Magnetotactic bacteria were discovered in 1975 by Richard Blakemore. He noticed that the bacteria from a mud drop placed on a microscope slide apparently migrated undirectionally along the magnetic camp lines, when a magnet was placed at the edge of the drop. In a couple of minutes, the entire population gathered at the "north" pole of the drop, but they spread as soon as the polarity of the magnet was reversed. These bacteria have a number of intracellular crystals (around 20) of iron oxides or greigite, each with Φ = 35-120 nm, called magnetosomes which are usually surrounded by a phospholipidic membrane. The bacterium uses them to distinguish between "up" and "down" in the terrestrial magnetic field and to navigate among the water layers, looking for optimal growing conditions. Due to the membrane, the biomagnets have a very high surface/volume ratio and they can be used as immobilization bases for bioactive substances (enzymes, antibodies, drugs). The bacterial magnetic crystals are synthesized by spirilla like *Aquaspirillum Magnetotacticum* and *Magnetospirillum* G*ryphiswaldense.*

The advantages of using biomagnets instead of artificial magnets are: the membranes prevent particle aggregation, maintaining large contact surface; the presence of some protein components in membrane allows a more efficient bounding of bioactive agents; the quite simple technique of conjugates' construction; the quantity of coupled bioactive agents is about 100 bigger; the conjugates are more stable; the activity lasts longer, in multiple uses; the conjugates' manipulation capacity with an exterior magnetic field; the biomagnetic particles are considered new and valid biological resources for nanobiotechnological applications.[50]

PREPARING DRUG CHARGED POLYMERIC MAGNETIC PARTICLES

The magnetic carriers designated for targeting, especially for local problems and more for tumor treatment, can be obtained in several ways, depending on the phase in which the drug is introduced in the preparation: either in the particle formation stage, that is the polymerization or reticulation process (most oftenly used procedure), or later, by enclosing (by interfacial absorption) into the magnet-polymer system. The polymer is used as protective biological layer to improve the particles' stability, especially to prevent particle agglomeration and protein absorption and so, to increase their efficiency in the internalization process through the target cells.

Drug immobilization in drug vectoring systems can be carried out by two methods: physical enclosing (encapsulation in microparticles; enclosing in liposomes) and chemical bounding (covalent coupling). Adding the drug to the previously obtained empty microparticles makes it able to be covalently coupled or sorption bounded. Sorption can be done either through drug diffusion in the polymeric network and creation of a solid solution, or through adsorption at the microparticle surface. Drug easier enclosing is explained by the existence of the surfactant polar macromolecules coupled to magnetic nanoparticles: these molecules form a filament micronetwork which favors creating some bounds with the drug molecules.

The complexation between aqueous the ferrofluid and the drug represents a molecular intimate phenomenon that can take place in at least three ways (Fig. 3): an electrochemical coupling is induced between the surfactant and drug molecules, if the two types of molecules present different electrical charges (Fig. 3a); a predictable bound between the surfactant and the drug molecules can be obtained adding a ligand, but this complexation modality presumes the introduction of an additional compound (Fig. 3b); drug molecules can penetrate and remain between the surfactant macromolecules placed on the surface of the magnetic nanoparticles (Fig. 3c).

Due to the ferrofluid's structure, the complexation method (c) coexists with method (a) and whenever the case, with method (b).

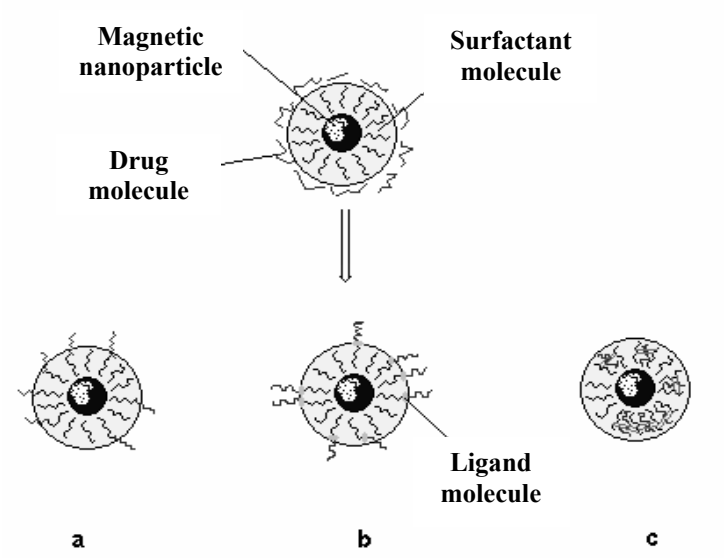

Fig. 3 – Methods of complexation drug substance – magnetic fluid.

The conjugates between magnetite and drug, especially antitumorals can have certain limitations due to the difficulty of controlling the release of the active principle and due to their small drug charge disponibility.

Hassan, 1992, used a combined technique of emulsion, reticulation and evaporation of the solvent in order to prepare chitosan with oxantrazole magnetic microspheres. Reticulation was performed with glutaraldehyde, the chemotherapy agent percentage was 3% and the particles' diameter was smaller than 1 μm.[15]

Ghassabian, 1996, prepared albumin magnetic microspheres (3-5 μm) in a classic way, similar to the one described by Widde, 1997. He used ferrofluid prepared according to Shimoiizaka's method (1976) and dexamethasone sodium phosphate as therapeutic agent.[17]

Nagano, 1997, prepares magnetic granules containing bleomycin hydrochloride as anticancerous agent, and a mixture of hydroxypropylcellulose and carbopol 934 as bioadhesive polymer. (600-710 μm).[12]

Bergemann, 1999, obtained magnetic nanoparticles and microparticles with epirubicin,[11] controlling their dimension through the speed of the thermic reaction and of the ferrous salts' concentration, which he later included in synthetic or natural polymers' matrixes, as well as poly(vinyl alcohol), polyacrylates, starch, pectin and alginates.

Alexiou, 2001, obtains ferrofluid particles covered with hydrophilic starch of 100 nm diameters, and which contain mitoxantrone ionically bounded to polymer's terminal groups like phosphate.[13]

Ramanujan, 2004, used iron oxide powder to prepare microspheres of 600 nm in diameter, through solvent evaporation. He used poly (lactic acid) as cover polymer, and teofiline (anhydrous 1, 3-dimethylxanthine) as drug.[18]

Arias, 2005 prepares the poly (ethyl-2-cyanoacrylate) nanoparticles with magnetite heart, that he charges with 5-fluorouracil. Hysteresis cycles upon magnetite and magnetite particles wrapped in polymer demonstrate that the polymeric membrane reduces the magnetic receptivity of the particles, but it maintains unaltered their light ferromagnetic character.[20]

Chen, 2005, used the radiation technique to obtain ferrogels formed of ferromagnetic nanoparticles (50 nm) encapsulated in poly(vinyl pyrolidone) microspheres and charged with bleomicyn A5 Hydrochloride. The ferrogels are new materials with magnetic nanocrystals included in a flexible polymeric network, having magneto-elasticity.[21]

Kohler, 2006, prepared poly(etylenglycol) magnetic nanoparticles in which he immobilized methotrexate. Covalent bonds, ester type, appear between the drug's glutamic acid and self-assembled monolayer of the polymer, bonds stable in intravenous conditions.[16]

Yang, 2006, uses the method of water/oil emulsion to prepare magnetic nanoparticles of poly(ε-caprolactone) in which he encapsulated gemcitabine and cisplatin as anticancerous drugs. The three components (magnetite, polymer and drug) were dissolved in dichloromethane in the presence of a stabilizer, then the mixture was ultrasonically treated and the solvent was evaporated, followed by centrifugation and redispersation.[10]

Yoshida, 2007, evaluates the force of a magnetic field applied in order to manage a system of drug magnetic release at the target, both *in vivo*, through mice's blood vessels, and in vitro, using a device made of glass tubes with pig blood.[14]

1. Magnetic carriers' characteristics

The complete characterization[51] of these particulate systems is needed to decide if using the nanocarrier system is favourable for certain *in vivo* applications. The physico-chemical properties that describe the nanoparticles are: particle dimension, toxicity, protein absorption capacity, surface hydrophobicity, charging speed, releasing kinetics, carrier system stability/discomposure, electroforetical mobility, porosity, density, crystalinity and molecular mass.

Particle dimension means particle's total diameter including the magnetic heart and its polymeric cover. The smallest body capillaries have the diameter of 4 μm; the microparticles with bigger diameters will be captured and held in the lungs, where they can produce embolization of arteries and lung capillaries. Mainly, particles smaller than 4 μm are rapidly cleared by Kupffer cells from liver (60-90%) and spleen (3-10%), as a result of opsonization (protein absorption) and "swallowing" by the reticuloendotelial system's macrophages. Particles with dimensions bigger than 100 nm are phagocytated in the liver cells, while those bigger than 200 nm are filtered through the venous sinuses of the spleen. At an intravenous administration of those with dimensions between 30 and 100 nm, the liver releases faster the bigger particles of the blood flux, compared to the smaller ones. Depending on the particles' size, their ingestion in the body is divided in phagocytosis and pinocytosis. Big particles can only be cleared by cells capable of phagocytosis, while smaller ones can be absorbed through pinocytosis by all types of cells. Thus, phagocitic activity increases proportionally with particle dimension.

Under physiological conditions particles bigger than 10 nm cannot penetrate the endothelium. Still, this permeability barrier can be lifted in pathological conditions, like tumor inflammation or infiltration, when 700 nm particles are allow to get into the system. An important conclusion may be pointed out: both *in vitro* and *in vivo* behavior of different dimension depending on their diameter.

Toxicity and biocompatibility. Besides the direct effects of the product, it is necessary to analyze the toxicity of the degradation products. In the case of magnetic particulate systems, toxicity *in vitro* can be studied on tumor cells. The investigation method consists in evaluating the quantity of surviving cells, the life time and tumor or cell growth. Citotoxicity is higher *in vitro* than *in vivo*, as live systems are capable of continuously releasing degradation products that generate toxicity.[52] The cover material of the magnetic particle should be a natural or synthetic biopolymer, as they chemically degrade at speeds that depend on the particle's size, surface properties, reticulation density and polymer's molecular mass. Without being exhaustive, albumin, dextran, chitosan, poly(lactic acid), poly(ethylene glycol) lactic and glycolic acids' copolymer can enter this category. Evaluation of acute toxicity of these biopolymers suggests that they cannot be responsible for the appearance of a toxicity effect in the magnetic particulate product.[52]

Polymers' biodegradability in human drug presumes breaking the polymer in resorbable (metabolizable) or excretable fragments. Degradation appears due to environmental factors (pH, temperature, solvents, and catalysts), chemical composition (water, oxygen, ozone, and halogenated compounds), electromagnetic radiation (visible light, ultraviolet light, γ radiations) and their associations.[53]

DRUG RELEASE FROM MAGNETIC PARTICULATE SYSTEMS

The type of bond established between magnetic polymeric particle and immobilized drug leads to different releasing mechanisms and speeds. Generally, covalent coupling leads to much lower releasing rate. Anyway, even if the drug is held in the particle as a solution or a solid dispersion, the releasing characteristics depend mainly on the polymer's degradation speed.[54] The general chart of the active principles releasing mechanisms from the vectoring polymeric systems of drugs is explained by Fig. 4.

Tests of drug release takes firstly place on lab animals, like mice, rats, cats, rabbits, dogs and even pigs. For instance, releasing epirubicin *in vitro*, in physiological conditions at 37^{o} C shows desorption with half-value life of 30 minutes. *In vivo* release shows a slowed desorption kinetics up to 45 de minutes. One reason for this behavior could be the slower diffusion of the drug through the vascular wall.[11]

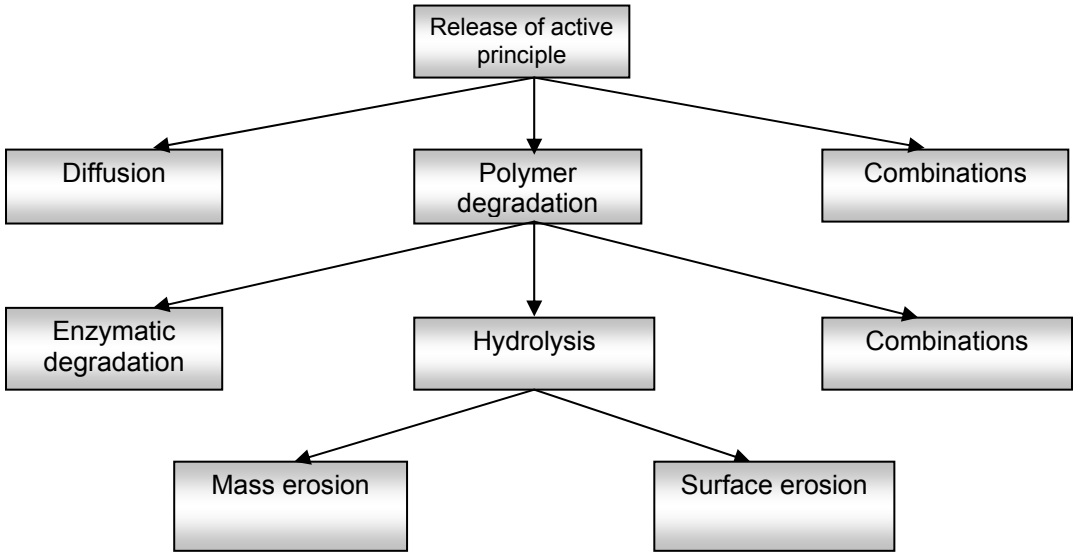

Fig. 4 – The mechanism of releasing active principles from the polymeric systems of drug vectoring.

Lubbe, 1996, tested in a magnetic field on mice and rats' bodies found the concentration of the magnetic fluid to which he attached a drug, cytokine. Two forms of therapy with the help of the magnetic fluids were: the tumor treatment by mechanical occlusion with high concentration of ferrofluid and controlled targeting of magnetic drug using small quantities of ferrofluid as a vehicle to focus the epirubicin in the tumor area. The magnetic fluid proved to be a safe agent that can be used in different ways to cure certain forms of local cancer together with magnetic field application. Bigger magnetic particles have better magnetic susceptibility and can be easily directed and retained at the target area.[22]

The author concludes that tested animals' physiological data help to estimate the complexity of the drug release systems on humans. Although they seem reliable, active targeting treatments need adjustments. While physiological characteristics and magnetic field cannot be too much modified, the ferrofluid-drug complex can be improved, as well as the administration method: arterial injection is less preferable compared to intravenous or direct injection in the tumor.[55]

Magnetic particles with doxorubicin were intra-arterially injected to pigs in the presence of the magnetic field. Regional distribution of drug carrier magnetic vehicles depends on the magnetic field, **Goodwin, 1999**.[56]

To overcome the problems of tumor's spatial configuration, **Kubo, 2000**[60] implanted permanent magnets in osteosarcoma solid tumor at hamsters, releasing the magnetoliposomes charged with cytotoxic drugs. This treatment method produced four times drug increase at the target area, compared to intra-venous administration without magnetic targeting.

Alexiou, 2001, used starch covered magnetic particles (100 nm) as biocompatible carriers for mitoxantrone. This system was intra-arterially injected in rabbits with tumors in the median area of the posterior members and directed with the help of an exterior magnetic field; after 15 days, complete remission of the tumor was noticed while the reference group, who did not receive the same treatment, presented an increase of the tumor.[13] The author studied the biodistribution of starch covered magnetic particles (100 nm), ionically coupled with different cationic therapeutical agents, through Iod^{123} marking, in magnetic field (0,6 T), at rabbits, and draw the conclusion that ferrofluid is accumulated in tumoral tissue two times more than in witness tissues under conditions.[59]

Kuznetsov, 2001, encapsulated muscular relaxants (diadone and diperone) in magnetic liposomes that he injected in kitties.[58] The target organ was one of the back paws, placed in a magnetic filed. The results were analyzed according to the muscle amplitude recorded after intravenously administration of the compound and compared with a reference outside the magnetic field. On injecting one dose of 115 μm/kg magnetic liposome encapsulated diadone, the muscular nerve amplitude is 70%, in the presence of magnetic field, and 15% without them, while at administering a dose of 20μm/kg diperone, under the same conditions, the amplitude decreases from 45% to 5%.

Saravan, 2004, prepared gelatin magnetic microspheres (5 μm) charged with sodium diclofenac (8.9%) and with magnetite (28.7%) which was injected intravenously, holding a magnet close to the target area. The study shows that 5.5% of the injected dose locates near the target organ, the rest are going to lungs, spleen and liver.[19]

Chen, 2005, prepared a ferrogels with 5 μm particles, of poly(vinyl pyrrolidone) with ferric oxide, in which bleomycin was immobilized as anticancer agent. When the compound is administered in the presence of an exterior magnetic field, a complete remission of the tumor is noticed. In the presence of the magnetic field, the ferrogels containing bleomycin slowly release the drug in the tumor area, which leads to drug concentration increase.[21]

Mykhaylyk, 2005,[57] prepared nanomaterials based on magnetite and dextran, which was intravenously injected in rats. The results show that, after destroying the blood-brain osmotic barrier, the dextran-magnetite nanodispersed compound can penetrate rats' brain tumor, as well as peritumoral tissue, in concentrations high enough to increase contrast imaging on IMR.

CLINICAL TESTING OF THERAPEUTICAL DRUGS

The magnetic field used to guide-vector the drug charged magnetized polymeric systems is generated by permanent magnets with high energy, made of rare earth, most of them containing neodymium. These can be arranged depending on the shape of every tumor, in their close neighborhood. The intensity of the magnetic field varies from 0.2 T to higher values and adjusts form case to case, together with the time length of its application. The magnets are placed at a distance of 0.5 cm of the tumor surface.[24]

Rotariu, **2005**,[61] evaluates, by a computer simulation modeling study, the concentration of the magnetic particles with nano or micro-metrical dimensions in tumors located at certain depths inside the body. Big dimensions cylindrical external magnets can lead magnetite particles of ≤ 1μm diameter through capillaries and tumoral small arteries. Particles with diameter bigger than 1μm are kept at a distance longer than 15 cm in tumor capillary state. The magnetic field generated in tumor by magnetic acicular implants focus the magnetic particles of ≤ 2 μm diameter in small surface regions (≈ 1cm), being suitable only for small dimensions tumors.

Classification of drugs immobilized in magnetic systems follows several criteria: according to the "targeting" grade: first, second and third targeting grade; according to the target area: organ, cell, subcell targeting; targeting type: passive and active targeting; side-directed targeting and side avoidance targeting; vehicle's transportation type: biochemical, biomechanical, biophysical and bioadhesive targeting; dependent and independent carrier.

Table 1 presents the main active principles and the polymeric systems that were immobilized:

Table 1

The main active principles and the polymeric systems those were immobilized

Release System	Activ Principle	References
starch	epirubicin	Lubbe, 1996[22]
poly (ethil-2-cianoacrilat)	5-fluorouracil	Arias, 2005[20]
gelatina	diclofenac sodium	Saravanan, 2004[19]
poly (vinil pyrrolidone)	bleomicyn A5 Hydrochloride.	Chen, 2005[21]
poly (lactic acid)	teofiline (anhydrous 1,3-dimethylxanthine)	Ramanujan, 2004[18]
starch	mitoxantrone	Alexiou, 2001[13]
hydroxypropylcellulose and carbopol 934	bleomycin hydrochloride	Nagano, 1997[12]
albumin	dexamethasone sodium phosphat	Ghassabian, 1996[17]
poly(vinyl alcohol), polyacrylates, starch, pectin and alginates	epirubicin	Bergemann, 1999[11]
poly(ε-caprolactone)	gemcitabine and cisplatin	Yang, 2006[10]
chitosan	oxantrazole	Hassan, 1992[15]
poly(etylenglycol)	methotrexate	Kohler, 2006[16]

Lubbe, 1996,[24] performed the first clinical experiments on human patients; epirubicin was chemically fixed to ferrofluid particles of 100 nm and both were covered by a polymer membrane (starch). Leading towards the target area was made by a magnetic field placed in the tumoral zone, at the surface of the skin. The conclusion of the study was that the patients tolerated well the administered ferrofluid infusion. Another experiment was focused on evaluating the potential for magnetic targeting of nanoparticles carrying doxorubicin upon hepatocelullar carcinomas by transcatheter lead through hepatic artery by IMR (**Wilson, 2004**).[62]

Lubbe, 1999, continued the clinical tests on humans, in order to improve the administration conditions and the therapeutical results. Arterial injecting is less recommended, intravenous and tumor direct injecting being preferred.[55]

Obstacles. Limitations to this kind of systems consisted of: (a) risk of embolization of blood vessels in the desired target region due to magnetic carrier accumulation, (b) difficulties in applying this technique on big animals, (due to the bigger distance between magnet and target area the drug after release is no longer sensitive at magnet's action, (c) the toxic answer of the magnetic carrier.[24]

Provocations. Patients with tumors located near the surface of the body or in the liver will be the first to beneficiate of this technique.

BIOMEDICAL APPLICATIONS OF MAGNETIC PARTICLES

The biomedical applications of the magnetic carriers cover many biomedical and biotechnological fields: separation of human blood components; blood vessel embolization, drug magnetic targeting, addition of antibody attached to superparamagnetic nanoparticle, locoregional radiotherapy, electromagnetically induced intratumoral hyperthermia, superparamagnetic contrast agents in nuclear magnetic resonance imaging, bioprocess acceleration.

1. Drug vectoring with magnetic carriers

Classical drug administration performed orally and intravenously, involves the drug transportation through the entire body (systemic distribution). A system of carriers that locoregionally releases the active substance has been developed in order to eliminate this distribution disadvantage. There are two types of drug targeting: passive and active. The passive one depends on the particle's dimension and surface, while active vectoring (drug delivery to target area) is performed with magnetic drugs, being influenced by the externally applied magnetic field. By applying magnetic targeting, a concentration of 70% of the drug dose in the target tissue is reached, with minimum interactions with the healthy cells and low toxic effect upon normal tissues; the concentration of the active substance in the target tissue increases to 80% using only a third of the drug dose used in conventional therapy. High microvascular permeability of the cancer tissues (almost 8 times bigger compared to the normal tissues) and much more accentuated diffusion (almost 33 times bigger) favor chemotherapy by magnetic targeting.

The application of magnetic therapy consists in three steps: (a) the preparation of the "magnetic drug"; (b) the introduction of this magnetic drug compound in the body (intravenously, implant or catheter), together with the application of a magnetic field on the problem area, (c) locoregional release of the compound drug substance.

In order to be used, magnetic drugs have to accomplish a series of conditions: to be safe and efficient, to carry a big quantity of drug, the active principle should be retained by the carrier until the release area in the vascular space at tumor level. The efficiency of such a system depends on a series of physical parameters of the magnetic drug and field: the dimension of the magnetic heart in the magnetic fluid, the characteristics of particles' surface, concentration of administered fluid, reversibility of drug-magnetic particle surface link, nonomogenity grade as well as intensity and application duration of the magnetic field.

Patient's physiological parameters must be correlated with the technical characteristics of the magnetic field and drug compound: weight, area, body height, blood volume, cardiac frequency, tumor volume and place, tumor blood flux.

The most recent studies have shown that ferrofluids are preferred for complexation as they have the following advantages: they are biocompatible, they attach the drug easier and they present a better *in vivo* stability under the magnetic field, they present intrinsic toxicity for tumor cells, even when the cytostatic lacks.

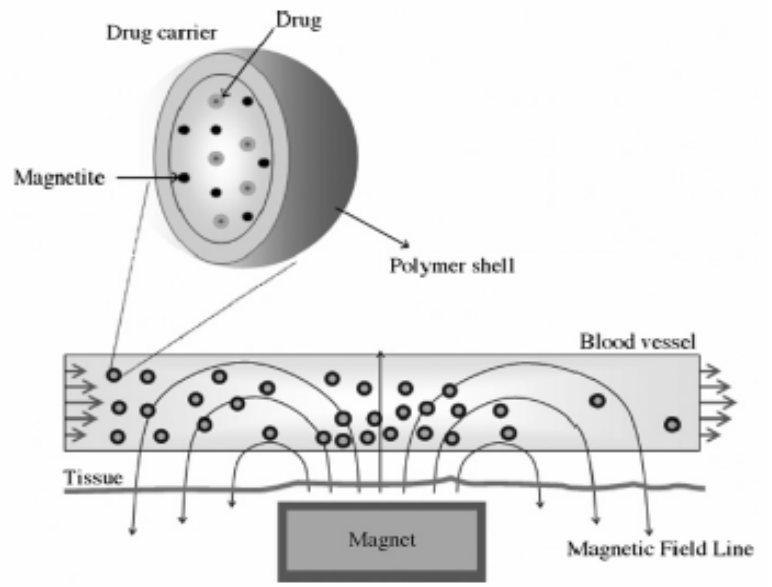

Fig. 5 – The schematic representation of drug magnetic vectoring: the magnetic carrier is directed to and releases the drug in the target area (Yang, 2006).[10]

2. Antitumoral therapy by electromagnetically induced hyperthermia

Intratumoral hyperthermia means increasing the temperature of the intratumorally disposed magnetic particles, due to electromagnetic energy absorption. When applying a relatively high frequency (0,5–10 MHz) and low intensity electromagnetic field (1,6–13 kA/m), for 2–4 hours, the temperature increases up to 47^0C, which modifies the functions and the structure of the neoplasic cells, destroying them. Studies have shown that ferrofluid nanoparticles distribute evenly in the tumor volume and they have an absorption rate of the electromagnetic energy higher than dispersed particles that behave like unique heating sources. Electromagnetically induced intratumoral hyperthermia method can be combined with magnetic targeting chemotherapy. The application was successfully tested on adenocarcinoma (prostate carcinoma), brain tumors (glioblastom) and residual tumors (after surgery).[2]

Known as malign tumor cure for more than 3,000 years, when the Egyptians burned superficial tumors, hyperthermia produces acute necrosis, protein coagulation or carbonization of the tissue put under high temperatures. Nanoparticles of iron oxides are candidates for cancer treatment as cellular necrosis support, using controlled and local heating of the tissues, based on vascularization particularities and blood supply of the tumors compared to the normal tissues. Generally, the blood supply in most solid tumors is frequently disorganized and heterogeneous, resulting in low perfused areas inside the tumor. Furthermore, this deficiency of blood circulation in tumor mass leads to weak heat dissipation, the tumor becoming "hotter" than normal surrounding tissues when it is thermally treated on the spot. Cells' hypoxia is accentuated due to vascularization deficiency and tumor blood supply, the cells lacking necessary nutrients for survival. Thus, the tumor cells, which already present an intrinsic tendency to anaerobe metabolism, increase this tendency, the quantity of lactic acid increasing in the cells and so, the local pH significantly diminishes. All these factors result in exposure and increased sensitivity of tumor cells at death by hyperthermia.

Hyperthermia also influences the activity of certain regulating proteins, kinase and cyclin, which lead to alterations of cell cycle that can reach apoptosis (cellular death – "suicide" scheduled by cell regulator system). This is why hyperthermia produces selective death of cells found in phase S of cellular cycle that resist radiotherapy. It is also interesting the fact that tumor cells exposed to thermal cure become easier recognized by the host's immune system, due to the alterations produced by the high temperatures in the structure of some receptor molecules in the cellular membrane. Hyperthermia is never used alone but in synergy, together with radiotherapy and chemotherapy (increasing the absorption grade of cytostatics at tissue level and the toxicity grade) in the multimodal cure of tumors.

For magnetic fluid hyperthermia, the capacity of absorbing the power radiated by an alternative field of lower frequency (<100 kHz) depends on the magnetic susceptibility composition as well as the magnetic properties of the target tissue (cell or tissue culture). To improve stability and biocompatibility, the particles are covered with polysaccharides and suspended in aqueous solvents.[44]

Magnetoliposomes are used due to their biocompatibility, as they can penetrate encephalic barrier, being used in interbrain expansive processes. Controlled release of drugs introduced inside the magnetoliposomes uses the same mechanism of external hyperthermia, which creates breaches in lipid membrane continuity of the magnetoliposome, allowing the "escape" of the drug kept inside.[63, 64]

3. Locoregional radiotherapy with PM

Locoregional radiotherapy by magnetic method tries to eliminate the problems generated by the classical one, which affects tissues and organs near the tumor. Selective irradiation is possible by magnetic carrier technique: MP are coupled through specific procedures with certain types of radioisotopes (like, ^{90}Y, with a maximum cytostatic ray of 10 mm), being introduced in the tumor area after that. An exterior magnetic field applied upon the tumor binds the isotope activated carriers, the radioactive emission appearing intratumorally only, up to fixed distance, depending on the emission type of the radioisotope or its proximity at most.

Preclinical studies were published regarding intracavitary and intraspinal locoregional radiotherapy, elaborating cure patterns for small, solid residual tumors, (left after surgery) or patterns of therapy for tumors found in places difficult to access.

Magnetic carriers find applicability in this field due to the following advantages: (a) the adequate attachment of high concentrations of radioisotope, (b) the obtaining of a large variety of dimensions and with desired distribution, (c) the possibility of adjustment of the stability grade, (d) the deposit in lyophilized state, (e) the activation (by radioisotope attachment) even before their use, (f) a further modification by attachment to antibodies, enzymes, proteins or saccharin acid derivates.[65] Introducing magnetic carriers in antitumor multimodal cure practice is one of the future's directions in this field.

4. Erythrocytes' magnetic separation

The essence of the magnetic separation process consists in the differentiated action of magnetic forces upon the components of a mixture, in comparison with forces of other nature. The concurrent forces to the magnetic ones are those derived from hydrodynamic interactions, dimensions of bodies are small and weight, inertia and friction are big. Depending on the orientation and value of this force group's, the components of a mixture are differently collected or deviated and friction appears. Independent of previously mentioned concurrent forces' activity, there are interaction forces between the components of the mixture, which, most often, have a negative effect in the magnetic separation process. An important factor represents the magnetic properties of the materials to be separated determined by their inner structure, where the electron magnetic moments have the main role. The red cells are the only blood cells that present intrinsic magnetic properties, even if they are very weak. Magnetic susceptibility of red cells with hemoglobin in reduced state is $3.88*10^{-6}$ (SI), value that belongs to the weak paramagnetic field. In very intense fields (B=2-8 T), red cells begin to attract each other due to the hemoglobin paramagnetism, the blood becoming thus more viscous. The erythrocytes separate through a discontinuous or a continuous procedure. The most adequate method for separating erythrocytes is magnetic separation in high gradient of magnetic field technique, considering the weak paramagnetic character and the small dimension of the red cells.

4.1. Magnetic bioseparation

There are two kinds of magnetic properties cells in nature: erythrocytes containing large quantities of hemoglobin and magnetotactical bacteria.[68] One or more nonmagnetic components of a mixture are coupled with magnetic entities. The attachment of the magnetic carriers is usually mediated by ligands with affinity towards different groups, which can interact with target cells from the cell surface. The new formed complex has magnetic properties and can be handled with a small magnetic separator placed nearby.

Cell separation process using magnetic carriers and separators has three steps: formation of the magnetic complex; analysis through different methods (chromatography, electrophoresis); detachment of the magnetic carrier from the target

cells, the carrier is taken from the suspension in a separator, and the separated cells go for analyses and applications.

The magnetic particles used for cellular separation must accomplish a couple of important criteria, such as chemical stability, non aggregation in the medium used for cellular separation, low magnetic remanence after exposure to magnetic field, as well as a size that prevents phagocytosis.

5. Contrast agents in magnetic resonance imaging

Nanoparticles with paramagnetic or super-paramagnetic properties can be used as contrast substances as they diminish relaxation times of the tissues in which they fixate. The following can also be used as contrast agents: macromolecules that have paramagnetic material ions as insertion material, magnetoliposomes, iron oxide supermagnetic nanoparticles, superparamagnetic nanoparticles covered with surfactant.[2]

Contrast agents from macromolecular compounds are made of biocompatible polymers complexed with paramagnetic ions. The most used ion is gadolinium, having the biggest number of unpacked electrons, increasing its paramagnetic properties.

6. Perspectives

Target drug magnetic vectoring is a field with great future, but, from research to clinical practice it is necessary to define some physical and magnetic properties of these systems. Firstly, the intensity (force) of the magnetic field should be carefully chosen and directed in order to deliver and focus the magnetic particles towards the target area. Thus, the magnetic particles should present an improved magnetic susceptibility in order to have a positive answer to the action of the magnetic field. Secondly, the magnetic particles should be small enough not to block the blood vessels through which they are guided towards the target organ. Furthermore, it must be taken into account the elimination method of the magnetic part after the therapeutic action out of the body, to prevent the local or systematic increasing toxicity on long or short term one. The particles should have uniform dimensions, to produce the equality of the magnetic capture for every magnetic sphere and thus constant drug content.[69] Besides the magnetic properties, the particle route throughout the body is a very important part for local and systematic toxicity for long or short term. The optimizing of pharmacokinetic characteristics of the target organ is also necessary, considering that a healthy, normal organ differs from a sick one.

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